SOME NEW SPECIES OF MINNESOTA ALGÆ WHICH LIVE IN A CALCAREOUS OR SILICEOUS MATRIX.

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(WITH PLATES VII–IX)

DURING the past three seasons there have been observed near Minneapolis several species of algæ which deserve attention from their peculiar manner of life, since they occupy not the surface but the interior of rock formations. They exist, therefore, under conditions of low illumination.

In the summer of 1894 a curious incrustation was noticed lining the sides of an old sunken tank which had formerly been used in connection with a rendering factory. The tank is situated on the eastern bank of the Mississippi river, two miles below this city. It is nearly forty feet square and six to nine feet deep, having a muddy bottom. The walls are of boards standing upright side by side and driven in like piles. The incrustation extends from the surface of the water downwards to a distance of perhaps three feet, where, becoming thin and scaly, it gradually disappears. Its thickness in 1894 was in the neighborhood of 2^{mm}. By the following year there was an increase to 6^{mm}, and in the present season it has attained an average thickness of 10^{mm}.

The crust covering the southwest side of the tank varies in color. Dull and bright æruginous, steel and brownish tints predominate, the two latter corresponding most nearly to the shades *cæsius* and *isabellinus* as given in Saccardo's *Chromotaxia* A close view of the surface shows it to be indented by very minute pores or depressions, which may be compared roughly to the markings on some of the corals and other lime secreting sea animals (*pl. VII*).

Subjected to chemical tests the incrustation is found to be made up almost wholly of calcium carbonate in the amorphous form, and organic material. It is exceedingly porous, absorbing water readily when dry, and is also very friable.

A microscopical investigation shows the presence throughout the stratum of species of algæ belonging to the Cyanophyceæ. Three species are found to be constant, a Dichothrix and two species of Lyngbya. Numerous diatoms and scattered cells of Glœocapsa are also present. The difference in color of the surface of the stratum is found to be due to the position of the above three species. When the Dichothrix appears on the surface a shade of light brown with a tinge of pink is given, or at times a bright cæsius blue. The Lyngbyas occasion the æruginous tints.

The calcareous matrix contains constantly an organism evidently fungal in character and corresponding in all respects to the chlamydospore-bearing filaments of *Pseudohelotium granulosellum* as figured by Brefeld.[1] The extraordinary occurrence of this fungus I am quite unable to explain, and its origin and development in the matrix must receive further study before anything of importance can be said about it.

In general the relative positions of the three algal species are as follows: the Dichothrix possesses the widest range and is the most abundant of the three. It occurs farthest from the light in the older portions of the lime stratum, as well as at the surface. Its arrangement is for the most part zonal. The filaments are parallel and stand perpendicular to the plane of the stratum. The large Lyngbya does not extend downward so far as the Dichothrix. It prefers, evidently, the area just beneath the surface of the crust, but at times it reaches the extreme surface. Its filaments form a tangled network. As a rule the small Lyngbya is found at the top of the matrix and immediately below the surface. The lower filaments consist of empty sheaths.

The growth on the remaining three sides of the tank shows

[1] Untersuchungen, Heft 10, *pl. 12, fig. 26.*

a somewhat different structure (*pl. VIII*). The incrustation just described now appears as the substratum, its surface being covered by the thalli of *Chætophora calcarea*, which was distributed as no. 11 in *American Algæ, Century 1*.

The thalli project from the substratum. They form somewhat globose mounds, or later these are confluent into sharp ridges or shelves parallel to the surface of the water. These shelves may be compared in shape to a Polyporus and are peculiar in construction. The upper portion consists of the Chætophora thalli proper, being in color a chlorophyll green; the substructure is made up of the blue-green species, notably the larger Lyngbya, which causes the bright purplish-blue color. Evidently, in the beginning, the Chætophora thallus is solitary, has a globose form, and stands out at right angles to the substratum, thus presenting one side to the direct light of the sun, while the opposite side is in the shade. The Lyngbya seizing the opportunity offered for additional room and indirect light soon forms a growth upon the under side of the Chætophora thallus. This in turn takes advantage of the support given by the Lyngbya, which it uses as a substratum, and takes an upright position to receive on all sides alike the direct sunlight. Thus the two plants develop, keeping pace with and aiding each other, until eventually the above mentioned structure is formed. It may be said that the Lyngbya forms a shelf upon which the Chætophora thallus may rest, or that the Chætophora makes of itself a screen for the protection of the Lyngbya. This is a distinct and somewhat peculiar form of symbiosis.

For a time it remained a problem why the Chætophora should be confined to the three sides of the tank, while the blue-green plants occupied also the fourth side. With some difficulty the position of the inlet of the tank was located. It was found that the water enters in the corner facing the south, that it flows out again at the west corner in a stream a foot in width, almost immediately disappearing in the ground. From this it appears that there is a current along the southwest side of the tank. Elsewhere the water, while not stagnant, is not subject to so much movement that it might be called running water. This then is the probable reason for the arrangement of the plants. The preference of Chætophora for quiet, pure water is known, while Lyngbya and Dichothrix flourish in waters either with or without a current.

The Chætophora thalli are strongly impregnated with lime and are hard, making decalcification necessary before examination under the microscope. The nature of the calcium carbonate in these thalli differs from that in the substratum. Here it appears in the form of crystal plates which, under the high power of the microscope, have a striated appearance. This results from the fact that they have running through them perforations or tubes corresponding in size and form to the Chætophora filaments. Branches of the Chætophora may be observed indeed entering these tubes and emerging at the opposite side of the crystal plate (*pl. IX, fig. 6*). If a longitudinal section be cut from a thallus and placed under the lens, the crystal plates being left intact, it will be seen that these pipes or tubes radiate from the center, following exactly the trend of the branches and for the most part containing the branches, though it is somewhat difficult to focus closely enough to observe the latter point with the thick crystals under the coverglass (*pl. IX, fig. 7*).

The Chætophora, as well as a thin growth of the blue-green plants, occurs on dead limbs which have fallen into the water from the trees on the banks. A few of the twigs taken out of the tank late in the autumn displayed after drying a violet tint on their under surface. This was caused by the presence of a small Chantransia, which, like the other algæ, was incrusted with lime. Its color when growing was probably green, since otherwise it would have been noticed before it was dry. It was accompanied by both the Lyngbyas, similar in all respects to those found in the stratum on the sides of the tank, with the exception that their cell contents had now assumed a bright violet color. In rare cases filaments were still found with the former æruginous tint, and some belonging to the larger species had a brown color. The change in color from æruginous to violet may have some connection with the approach of cold weather. It was also noticed that the sheath of the larger Lyngbya had become corrugated or roughened and somewhat wider.

It is thought that these five algæ, which have just been described, are capable, either alone or in combination, of causing the precipitation of calcium carbonate. If the deposit is not formed in this way, it must be because the water contains a large quantity of calcium carbonate which is laid down as the result of evaporation. In this case these algæ have become adapted to a life within a calcareous envelope. As a matter of fact the water is not rich in carbonates. An analysis kindly made for me by Professor G. B. Frankforter shows the following results:

Total solids, - - - -	36 grains per gallon.
Calcium carbonate, - - -	18 " " "
Calcium sulphate, - - -	17.5 " " "
Sodium chloride, - - - -	trace.
Magnesium sulphate, - -	trace.

Another fact in favor of the supposition that the plants act as agents in the deposition is that the precipitation of calcium carbonate takes place only where the plants occur, and not indiscriminately upon every object exposed to the action of the water. A dead branch of a tree, after being in the water a year, was taken out to be preserved. The top and sides, as it lay in the water, were covered with a luxuriant growth of the several blue-green algæ and the Chætophora. On its under shaded surface the algæ would not live, nor was there a trace of lime to be found there. Again, the water has formed a ditch around the outside of the tank, deep and narrow, and therefore dark. For the latter reason no algæ grow on the back of the boards, and no deposit is formed there, though they are washed by the same water that circulates through the interior of the tank.

In certain waters at Mammoth Hot Springs, Yellowstone Park, where tourists suspend articles to be incrusted, the deposit coats the entire surface of the object. In this case the lime is deposited through exposure to the air of water containing a great abundance of calcium carbonate, and not through the agency of algæ.

It has not been proved that any one of the blue-green species or the Chantransia is able by itself to produce a separation of the carbonate, but two facts show the Chætophora to be independent of the others in its secretion of lime; first, its thalli are not engulfed in the substratum, and, second, the calcium carbonate is deposited in crystal plates instead of amorphous particles.

Until recently the only additional inhabitants of the tank have been a species of moss, a Fontinalis, which formed a rich growth all over the bottom of the tank, and the little fresh-water shrimp, *Gammarus pulex*, which is present in exceedingly great numbers. During the latter part of the recent summer, however, the water has appeared less pure, and a heavy growth of Spirogyra spread over the surface. The blue-green algæ remain unchanged, but the Chætophora has not thrived so well. It must be noted that not all the plants growing in the tank possess the ability to cause the precipitation of lime. Neither the moss nor the Spirogyra show a tendency to do so.

In preparing a slide of the above material it is a good plan first to soak a piece thoroughly in water, then cutting off a thin section with a scalpel place in a dish of diluted hydrochloric acid and warm gently. When the bubbles of CO_2 cease forming, it can be mounted in water or glycerine. Before putting the cover glass in place, it is well to tease apart the filaments with needles, for the section is likely to be too thick for perfect transparency.

Dichothrix calcarea Tilden, Am. Alg. Cent. II. no. 165. 1896. (*pl. IX., figs. 1–3*)—In extended strata either on surface of calcareous matrix, giving it then a brownish or sometimes a light æruginous tinge, or in layers throughout the matrix. Filaments 9–12.5μ in diameter, erect, not rigid; pseudobranches appressed; sheath rather thin, hyaline; trichomes brown, sometimes æruginous, up to 10μ in diameter, for the most part moniliform in lower portions, tapering to a point; articulations in lower portion of filament equal in length to diameter, shorter in upper portions; heterocyst basal, globose or depressed globose, diameter equal to or a little smaller than that of filament.

This plant does not seem to be very near any of the species of Dichothrix as described by Bornet and Flahault. The filaments are strongly agglutinated, and this with the moniliform character of the trichomes make it peculiar.

LYNGBYA MARTENSIANA **calcarea** Tilden, Am. Alg. Cent. II. no. 178. 1896. (*pl. IX., fig. 4*)—In extended strata throughout upper portions of calcareous deposit. Filaments elongate, straight, flexible, somewhat unequal in size, average 6.5–7.5μ in diameter; sheath very distinct, hyaline, smooth or rough; trichomes dull æruginous, violet, or rarely brown, frequently interrupted, not constricted at joints, not or very rarely attenuate at apex, 5–6.5μ in diameter; articulations 2–3 times shorter than diameter, average 2.5μ long; dissepiments often inconspicuous or marked with granules; apical cell rotund; calyptra none.

L. martensiana has been found only in thermal waters. The temperature of the water in the tank is 12° C. during the summer. The filaments of the species are somewhat larger and the articulations shorter than those of the variety, but otherwise the points agree very well.

Lyngbya nana Tilden, Am. Alg. Cent. II. no. 179. 1896. (*pl. IX., fig. 5*)—In extended strata on or near surface of deposit. Filaments 1.9μ in diameter, straight; sheath delicate, hyaline, smooth; trichomes very pale steel color becoming violet later in the season, not constricted at the joints; articulations 1.6μ in diameter, quadrate or 1.5 times the diameter in length; apical cell rotund.

In Gomont's monograph there are but four species of Lyngbya described whose size will permit of comparison with *L. nana*. Of these *L. Lagerheimii* is easily distinguished from it by the spiral filaments; *L. rivulariarum* by the constriction at the dissepiments, length of the articulations and habit of growth; *L. ochracea* differs in the peculiar character of its stratum; *L. purpurea* agrees more nearly than the others. The measurements are alike, the joints show no constriction. The violet color, however, which in the last species appears to be constant, is peculiar to a certain stage only of *L. nana*. The habit and habitat, likewise, distinctly separate the two.

Chætophora calcarea Tilden, Am. Alg. Cent. I. no. 11. 1894 (*pl. VIII.* and *pl. IX., figs. 6–7*)—Thalli globose, subglobose, or confluent into ridges, encrusted with lime. Lower cells 9μ in diameter, 3–5 times as long; upper cells 8–12.5μ in diameter, two times as long; articulations distinctly contracted at joints; terminal cells usually rather blunt, sometimes ending in very long articulated setæ.

The presence of lime in the thallus has been employed as a varietal character in the genus Chætophora in two instances, viz.: *Chætophora cornu-damæ* (Roth) Ag. var. *crystallophora* Kg. and var. *incrustans* Rabenh. An examination of herbarium material comprised under eight species indicates the presence of lime in quantity in twenty-seven out of forty-five cases. Eighteen specimens show no trace of the substance. Out of twenty specimens of *C. cornu-damæ*, ten showed strong indications of lime, four of these being of the var. *crystallophora*, and two being of the var. *clavata*. Eight out of the nine specimens of *C. tuberculosa* were encrusted with lime.

Kjellman's specimen from the polar sea, *C. pellicula*, said to form a crust 200–300μ in thickness, is in all probability a lime secreting plant.

C. calcarea and a plant nearly related to this genus, *Stigeoclonium flagelliferum* Kg. (*Pilinia diluta* Wood), both studied in this laboratory, possess the capacity of secreting lime to a remarkable degree. In both the calcium carbonate is deposited in the form of crystal plates, which are penetrated by the filaments and branches of the plant.

Taking these facts into account, it would seem that the presence or absence of lime in Chætophora thalli should be regarded as a factor in the determination of the species.

CHANTRANSIA PYGMÆA (Kg.) Sirodot, Les Batrachospermes 244, 245. 1884. Am. Alg. Cent. II. no. 112. 1896 (*pl. IX., fig. 8*)—Stratum very thin, when dry forming a violet-colored calcareous crust on lower shaded surface of dead twigs. Filaments straight; branches erect, sometimes appressed to stem, apices somewhat attenuate; articulations 11–12μ in diameter, in general 2–3 times the diameter in length; branches bearing sporules short, situated in upper portion of the plant; sporules in general 2–3 upon a branch.

The description of the asexual form of *Batrachospermum crouanianum*, as given by Sirodot, seems to cover fairly well the characters of the above plant. But, so far as is known, the capacity for secreting lime has not hitherto been noted in connection with this species.

A still more curious alga is one which inhabits the white sandstone cliffs at a point where Minnehaha creek flows into the Mississippi river. The rock presents no trace of plant life on its outer face, which has the usual appearance and light gray color of weathered white sandstone. But small pieces broken off and held up to the light show fine colorless threads hanging from the inner side of the fragments. These are filaments of a Schizothrix. The plant is found at least one-half inch from the outer surface. The amount of light received by it is necessarily extremely small, for the reflecting surfaces offered by the crystals are very numerous in such a thickness of stratum.

There is some difficulty in extracting the algal threads from the sand grains. The only satisfactory method is to moisten a bit of the material and place it under the low power of the microscope. The grains can then be removed with a needle, allowing the filaments to remain. It is necessary to use a $\frac{1}{16}$ oil immersion lens in order to observe the dissepiments.

Schizothrix rupicola Tilden, Am. Alg. Cent. II. no. 175. 1896 (*pl. IX., fig. 9*)—No definite stratum. Filaments 9.6–16μ in diameter; sheath cylindrical, rough, for the most part hyaline, sometimes brownish and much lamellated; trichomes pale æruginous, one to many in a sheath, not constricted at joints, 3.5–4.8μ in diameter; articulations 1–1.5 times as long as wide, 5–8μ long; dissepiments for the most part invisible; apical cell truncate conical or rarely somewhat attenuate.

Bare and dry sandstone cliffs, not on surface of rock, but extending within the interior to a distance of at least 10–15^{mm}. Collected by Professor C. W. Hall, Sept. 28, 1896.

S. rupicola agrees with *S. Friesii* in the diameter and length of the articulations and in the shape of the apical cell; but the trichomes do not display the constriction at the dissepiments which is so evident in the latter species, nor are the dissepiments themselves so conspicuous, it being nearly impossible to observe them even under the $\frac{1}{16}$ oil immersion lens. Furthermore, it does not possess a coarsely granulate protoplasm, which characteristic Gomont proposes as a test for recognizing the species. It also differs in habitat. *S. rubella* is likewise similar in the matter of dimensions and in the non-constriction of the joints. It disagrees, however, in having distinct dissepiments, coarsely granulate protoplasm, and forming a reddish lime incrusted stratum on wet rocks. In the morphological characters of the filament, *S. rupicola* approaches *S. penicillata*, but is distinguished from it by the entirely different habit.

The plants described in this paper as inhabiting the limestone crust were collected and studied at intervals during a period of two years. I wish to thank Professor MacMillan for the help he has given me in the work.

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EXPLANATION OF PLATES VII–IX.

PLATE VII.

Photograph of deposit on one of the planks from the southwest side of the tank. The crust is made up of the Dichothrix and the two Lyngbyas.

PLATE VIII.

Photograph of deposit on a plank from another side of the tank showing Chætophora thalli.

PLATE IX.

FIG. 1. Filament and pseudobranch of *Dichothrix calcarea*.

FIG. 2. Young filament of the same.

FIG. 3. Group of branches of the same.

FIG. 4. Group of filaments of *Lyngbya martensiana calcarea*.

FIG. 5. Group of filaments of *Lyngbya nana*.

FIG. 6. Filaments of *Chætophora calcarea* penetrating crystal plates.

FIG. 7. Section through thallus of *Chætophora calcarea* showing arrangement of crystal plates.

FIG. 8. Portion of a plant of *Chantransia pygmæa* showing branch bearing sporules.

FIG. 9. *Schizothrix rupicola*, with sheath containing three filaments.